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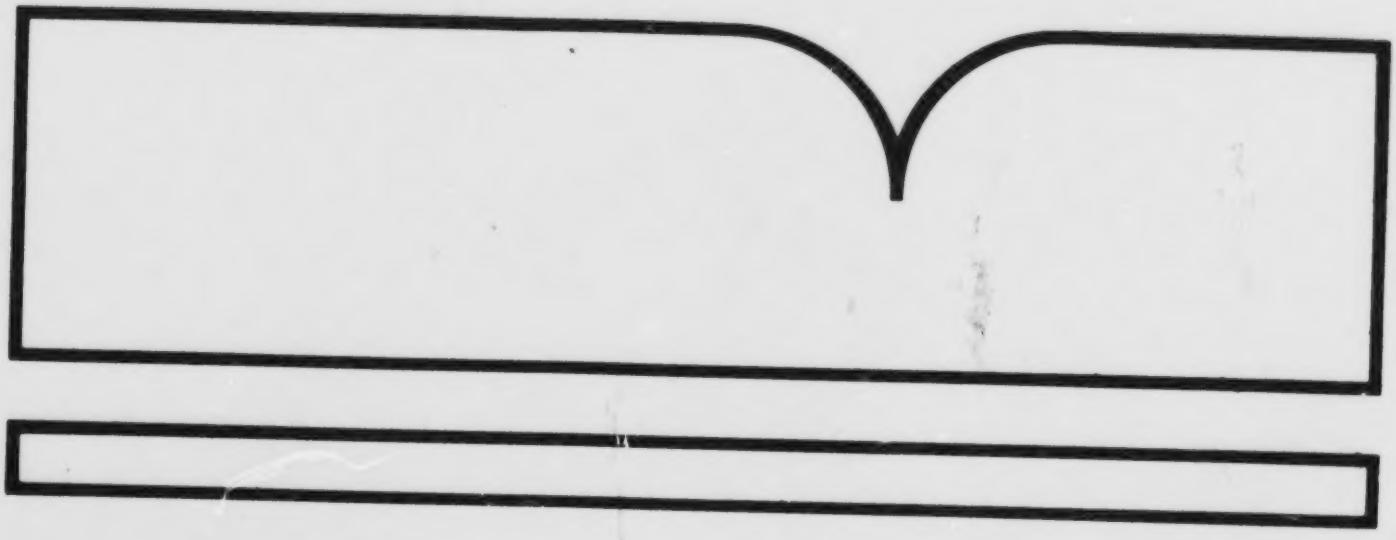

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EVALUATION OF CAPILLARY SYSTEMS FOR THE ANALYSIS OF ENVIRONMENTAL EXTRACTS

by

Thomas A. Bellar
Paul Stemmer
James J. Lichtenberg
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

Project Officer
Thomas Bellar
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

Environmental Monitoring and Support Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

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The quantitative and qualitative aspects of splitless and on-column capillary column injectors for the analysis of environmental extracts for priority pollutants are investigated. Precision data are presented for a wide variety of compounds introduced into a splitless injector by manual and totally automated injection practices. Similar studies are performed using manual injections into an on-column injector. Other studies include capillary system reactivity toward liable analytes, column selection, and the identification of the limi tations of current capillary column systems with respect to the analysis of complex extracts. Sufficient experimental evidence is documented to support the need for a unique quality assurance program, not associated with packed column systems.

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory - Cincinnati, conducts research to:

- Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
- Investigate methods for the concentration, recovery, and identification of viruses, bacteria and other microbiological organisms in water; and, to determine the responses of aquatic organisms to water quality.
- Develop and operate an Agencywide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
- Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This report investigates the possibility of updating existing organic analytical methods to take advantage of current developments in the field of capillary column gas chromatography. In addition to identifying sensitive parameters, a sound quality control program is outlined which will help to insure the quality of analytical data obtained from the analysis of environmental extracts by capillary column gas chromatography.

Robert L. Booth

Robert L. Booth, Acting Director
Environmental Monitoring and
Support Laboratory - Cincinnati

ABSTRACT

The quantitative and qualitative aspects of splitless and on-column capillary column injectors for the analysis of environmental extracts for priority pollutants are investigated. Precision data are presented for a wide variety of compounds introduced into a splitless injector by manual and totally automated injection practices. Similar studies are performed using manual injections into an on-column injector. Other studies include capillary system reactivity toward liable analytes, column selection and the identification of the limitations of current capillary column systems with respect to the analysis of complex extracts.

Sufficient experimental evidence is documented to support the need for a unique quality assurance program, not associated with packed column systems.

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LIST OF ABBREVIATIONS AND SYMBOLS

BP	boiling point
cm	centimeter
cm/sec	centimeter per second
^{o}C	degree Centrigrade
df	capillary column film thickness
ECD	electron capture detector
FID	flame ionization detector
GC	gas chromatography
I.D.	internal diameter
K-D	Kuderna-Danish
m	meter
MDL	method detection limit
µL	microliter
mL	milliliter
mm	milimeter
MS	mass spectrometry
ng	nanagram
PCBs	polychlorinated biphenyls
RSD	relative standard deviation
t	students t' test value

SECTION 1

INTRODUCTION

Over the past years an important in-house and extramural effort of the Environmental Monitoring and Support Laboratory - Cincinnati has been to develop analytical methods for the analysis of synthetic organic compounds contained in a wide variety of waters. Most recently the analytical protocol for fifteen separate methods, commonly called the 600-series methods, were developed for 103 specific organic compounds and nine product mixtures, commonly referred to as the "Organic Priority Pollutants." Each of the organic priority pollutants are listed in Table 1 along with the 600-series number identifying the proposed method. Part of the criteria required for the original investigative design was whenever possible, the finalized methods utilize both analytical equipment common to most analytical laboratories as well as established environmental methodology currently required in the National Pollutant Discharge Elimination Standards (NPDES) program[1] and the National Interim Primary Drinking Water Regulations (NIPDWR). The purpose of these requirements being as the methods are adopted by the Agency minimal start-up problems should be experienced by established analytical laboratories.

For each of the 15 methods, two basic extraction principles are used to separate the analytes from the water matrix, either purge and trap[4] or liquid/liquid extraction[1]. All but two of the methods employ packed-column gas chromatography for separation and detection. Other than the two extraction procedures, the primary difference between each of the methods are the clean-up and the gas chromatographic conditions required to resolve the analytes from one another and from common interferences.

Table 1
List of Organic Priority Pollutants

Parameter	EPA Method Number GC[a]	GC/MS	HPLC[c]
Acenaphthene	610	625	610
Acenaphthylene	610	625	610
Acrolein	603	624	-
Acrylonitrile	603	624	
Aldrin	608	625	610
Anthracene	610	625	610
Benzene	602	624	-
Benzidine		625	605
Benzo(a)anthracene	610	625	610
Benzo(a)pyrene	610	625	610
Benzo(b)fluoranthene	610	625	610
Benzo(ghi)perylene	610	625	610
Benzo(k)fluoranthene	610	625	610
Benzylbutyl phthalate	606	625	
α-BHC	608	625	-
β-BHC	608	625	-
γ-BHC	608	625	-
Δ-BHC	608	625	-
Bis(2-chloroethyl) ether	611	625	-
Bis(2-chloroethoxy) methane	611	625	-
Bis(2-chloroisopropyl) ether	611	625	-
Bis(2-ethylhexyl) phthalate	606	625	-
Bromodichloromethane	601	624	-
Bromoform	601	624	-
Bromomethane	601	624	-
Carbon tetrachloride	601	624	-
4-Chloro-3-methylphenol	604	625	-
Chlorobenzene	601, 602	624	-
Chlordane	608	625	-
Chloroethane	601	624	-
2-Chloroethylvinyl ether	601	624	-
Chloroform	601	624	-
Chloromethane	601	624	-
2-Chloronaphthalene	612	625	-
2-Chlorophenol	604	625	-
4-Chlorophenylphenyl ether	611	625	-
Chrysene	610	625	610
p,p'-DDD	608	625	-
p,p'-DDE	608	625	-
p,p'-DDT	608	625	-
Dibenzo(a,h)anthracene	610	625	610
Dibromochloromethane	601	624	-
1,2-Dichlorobenzene	601, 602, 612	624, 625	-
1,3-Dichlorobenzene	601, 602, 612	624, 625	-
1,4-Dichlorobenzene	601, 602, 612	624, 625	-
3,3'-Dichlorobenzidine	—	625	605

Dichlorodifluoromethane	601		
1,1-Dichloroethane	601		–
1,2-Dichloroethane	601	624	–
Dichloroethene	601	624	–
trans-1,2-Dichloroethene	601	624	–
2,4-Dichlorophenol	604	624	–
1,2-Dichloropropane	601	625	–
cis-1,3-Dichloropropene	601	624	–
trans-1,3-Dichloropropene	601	624	–
Dieldrin	608	624	–
Diethyl phthalate	606	625	–
2,4-Dimethylphenol	604	625	–
Dimethyl phthalate	606	625	–
Di-n-butyl phthalate	606	625	–
Di-n-octyl phthalate	606	625	–
2,4-Dinitrophenol	604	625	–
2,4-Dinitrotoluene	609	625	–
2,6-Dinitrotoluene	609	625	–
Endosulfan-1	608	625	–
Endosulfan-2	608	625	–
Endosulfan sulfate	608	625	–
Endrin	608	625	–
Endrin aldehyde	608	625	–
1,2-Diphenylhydrazine		625	–
Ethylbenzene	602	625	–
Fluoranthene	610	624	–
Fluorene	610	625	610
Heptachlor	608	625	610
Heptachlor expoxide	608	625	–
Hexachlorobenzene	612	625	–
Hexachlorobutadiene	612	625	–
Hexachlorocyclopentadiene	612	625	–
Hexachloroethane	612	625	–
Indeno(1,2,3-cd)pyrene	610	625	–
Isophorone	609	625	610
Methylene chloride	601, 618	625	–
2-Methyl-4,6-dinitrophenol	604	624	–
Naphthalene	610	625	–
Nitrobenzene	609	625	610
2-Nitrophenol	604	625	–
4-Nitrophenol	604	625	–
N-Nitrosodimethylamine	607	625	–
N-Nitrosodi-n-propylamine	607	625	–
N-Nitrosodiphenylamine	607	625	–
PCB-1016	608	625	–
PCB-1221	608	625	–
PCB-1232	608	625	–
PCB-1242	608	625	–
PCB-1248	608	625	–
PCB-1254	608	625	–
PCB-1260	608	625	–

Pentachlorophenol	604	625	-
Phenanthrene	610	625	610
Phenol	604	625	-
Pyrene	610	625	610
2,3,7,8-Tetrachloro-dibenzo-p-dioxin		613	-
1,1,2,2-Tetrachloroethane	601, 618	624	-
Tetrachloroethene	601, 618	624	-
Toluene	602	624	-
Toxaphene	608	625	-
1,2,4-Trichlorobenzene	612	625	-
1,1,1-Trichloroethane	601	624	-
1,1,2-Trichloroethane	601	624	-
Trichloroethene	601	624	-
Trichlorofluoromethane	601	624	-
2,4,6-Trichlorophenol	604	625	-
Vinyl Chloride	601	624	-

[a] Method number using conventional gas chromatographic detectors
[b] Method number using gas chromatography/mass spectrometry
[c] Method number using high performance liquid chromatography

As these methods were being developed and validated, significant advances were being made in the application of capillary column gas chromatography to the analysis of environmental extracts. These advances include: the commercial introduction of glass and fused silica capillary columns, splitless injectors, on-column injectors, user-programmable data systems, and a new generation of automated gas chromatographs especially designed for capillary columns. Upon introduction by manufacturers of gas chromatographic equipment, these tools appeared so powerful that many laboratories replaced or updated their packed column instrumentation in anticipation that the Agency would accept these advances as equivalent alternatives to packed column gas chromatography.

The purpose of this investigation was to evaluate commercially available capillary columns, injectors, and automated systems, to determine their performance as either equivalent or superior to the packed column systems currently required for the analysis of extracts obtained from liquid/liquid extraction procedures;[1,2] to identify problem areas and attempt to resolve them by recommending updates of the quality control or maintenance programs described in the existing methods; and finally, to determine if analytical conditions can be chosen so that a number of the 600-series methods can be combined so that a single capillary column analysis will provide the same quality of information provided by several separate packed column determinations. The primary intent of this study was to address the needs of the Office of Drinking Water (ODW) and to assist the Alternative Test Procedure Program (Equivalency) concerning requests to substitute capillary systems for the packed column systems currently required by ODW.

SECTION 2

EQUIPMENT

A Hewlett-Packard 5730 gas chromatograph equipped with dual flame ionization detectors (FID), linear electron capture detectors (ECD), and a Hewlett-Packard 18740A capillary column control was initially used for this study. It was found that the 18740A capillary column control adversely affected the performance of the capillary system because of intermittent internal leakage. An improved model 18740B capillary column control was purchased and found to generate acceptable chromatograms when used in conjunction with an optimized column and detector. Studies involving column selection were performed using this instrument. A Hewlett-Packard 5880 gas chromatograph equipped with a Hewlett-Packard 7672 autosampler, dual FID, dual ECD, a Hewlett-Packard Level 4 data system, dual splitless injectors, and a Model 19320D on-column injection assembly was used to determine the quantitative aspects of splitless and on-column injections. Although several different capillary columns were used early in this study, all of the qualitative and quantitative data listed in Tables 3 through 16 were obtained from a fused silica 30M, 0.32 mm ID, 0.25 µm film thickness polymethyl (5% phenyl) siloxane bonded phase capillary column.

SECTION 3

EXPERIMENTAL

The gas chromatographic operating conditions were initially adjusted according to the manufacturer's recommendations, using an ECD or FID. Complex mixtures of polychlorinated biphenyls (PCBs), organochlorine pesticides, and other priority pollutants were analyzed to select the proper columns and to further optimize chromatographic separations. Automated and manual splitless injection operations and techniques were evaluated by injecting standard solutions of organochlorine pesticides and comparing the resulting data to on-column injections. All data reported herein were obtained using area measurements generated solely by the data system.

Column Selection

Based upon a review of scientific literature, operators' manuals, and product advertisements, it appeared that wide-bore 0.25 µm film thickness (df) fused silica columns with lengths between 20 and 30 m would provide the chromatographic column performance necessary for analyzing complex environmental extracts over a moderate concentration range within a reasonable analysis time. Columns with these dimensions do sacrifice resolution; therefore, a series of experiments were performed using fused silica columns of varying lengths, internal diameters, and film thickness to validate the literature review. Columns with varying polarity were also tested to determine which commercially available liquid phases are best suited for resolving complex mixtures of priority pollutants. Each column was operated well within operating temperatures recommended by the manufacturer. Helium was used as the carrier gas and the linear gas velocity through the column

adjusted to 29 ± 5 cm/sec (measured with CF_3Cl at 90°C). An ECD operated with a 5% methane in argon sweep gas or a FID operated with helium sweep gas was used as detectors. Proper installation of each column was assured by routinely analyzing Grob polarity test mixtures[4] or, whenever possible, comparing system performance chromatograms to test chromatograms supplied by the column manufacturer. For electron capture operations, system performance was monitored by routinely analyzing mixtures of thermally labile organohalides.

Complex mixtures of PCBs, organochlorine pesticides, and other priority pollutants were injected into each column using the splitless mode of injection. One microliter (+0.8 µL needle volume) of sample was injected into a 220°C (2.0 x 78 mm) glass injector sleeve. The volatilized sample was injected into the column for 30 seconds, followed by an exhaustive backflushing of the injector. During injection, the column temperature was maintained at 90°C, then rapidly programmed to about 130°C, followed by a gentle program to 20° lower than the maximum recommended operating temperature of the column. Because of the initial column temperature, injection volume, and the boiling point of the solvents used under these conditions, sample reconstitution does not take place on the column by the "solvent effect."[5] Reconstitution was accomplished by "cold trapping," a condition where injection is made into the column while it is maintained approximately 150° below the elution temperature of the analyte. Sharp symmetrical peaks were obtained for representatives of each of the PCB homologs, organochlorine pesticides, and most other priority pollutants. Tests were also performed on low boiling priority pollutants. For these

compounds the initial column operating temperature was adjusted to obtain reconstitution by the "solvent effect" (approximately 10°C below the boiling point of the solvent).

Columns 0.32 mm I.D. x 30 m long with a 0.25 µm df provided sufficient separation to resolve extremely complex mixtures of priority pollutants with analysis times less than 45 minutes. Doubling the column length to 60 m improved the resolution between adjacent peaks only slightly and extended the analysis time so significantly that such column lengths did not justify the increased analysis time. Thick-film columns (1 µm df), often used because of their large sample capacity, increased the analysis time and provided inadequate separations between many priority pollutants. Five different liquid phases were evaluated. They were, in order of increasing polarity: methyl silicones (including OV-1 and OV-101); SE-54; OV-17; and Carbowax 20M. The analysis time for organochlorine pesticides on the Carboxwax 20M column proved too long for the column to be of any value. The OV-17 and SE-54 columns provided near equivalent resolution, superior to that obtained using a OV-1 or OV-101 column. Based on observations of resolution, analysis time, sample capacity, and peak geometry for a wide cross section of priority pollutants, a 30 m x 0.32 mm I.D. 0.25 µm df fused silica open tubular columns coated with liquid phases with polarities similar to OV-17 were selected for additional experimental work. It was concluded that the methyl silicone columns could be used as a confirmatory column.

After a reasonable column conditioning period, hexane reagent blank analyses were performed with each test column programmed from 90°C to 250°C (Carbowax 20M 90°C to 210°C). Limit of detection estimates were performed

where ECD or FID was operated at a sensitivity sufficient to detect analytes well below the reported[6] packed column method detection limits (MDL). The purpose of the test was to determine if low level noise, signals from column substrate bleed, ghost peaks, or interferences originating in the sampling system would preclude the use of capillary systems for trace analyses. It was found that, over the temperature program extremes studied, the detector noise level remained constant (<1% of full scale) and the baseline did not change more than 20% of full scale, establishing that ECD and single-column FID temperature programmed analyses are feasible using capillary columns for the measurement of environmental extracts normally associated with carefully optimized isothermally operated packed column systems.

"Ghost" peaks due to bleed from the splitless injector "O" ring seals and the septum produced tailing peaks throughout the chromatogram. These peaks were minimized by using preconditioned septums and "O" rings and by maintaining the column above 180°C until just before the analysis. The peaks, although annoying, were easily identified in the chromatogram because of their tailing nature. New microsyringes with removable needles when used with the autosampler caused a multitude of early eluting peaks in electron capture chromatograms. Permanently cemented needle syringes conditioned rapidly yielding acceptable blank analyses after only a few injections. Removable needle syringes never produced acceptable electron capture blank analyses within the average life of the needle (three weeks). Samples were stored in and sampled by the autosampler from 2 mL Teflon-lined, septum-sealed screw-cap glass vials. It was found that if the Teflon liner is properly installed toward the sample and is unpunctured, then solvent blanks and organochlorine pesticide solutions can be safely stored at room temperature for at least 48

hours. If the seal is inverted or the Teflon layer is pierced by the autosampler, then after about one and one-half hours extraneous peaks appear in the electron capture chromatograms. Depending upon the volatility of the solvent, as soon as two hours after the Teflon is pierced, analytes appear to increase in concentration due to solvent evaporation.

System Reactivity for Analytes

Early in this study, retention data were gathered for each of the single component priority pollutant pesticides. Those pesticides known to be thermally labile or unstable were analyzed individually, while those considered to be stable were analyzed as simple mixtures containing several easily resolvable components. General system performance and retention data were gathered for each pesticide by observing peak geometry and peak maxima. The absence of extraneous peaks, peak tailing, and abnormal peak widths indicated that the system performance was not only acceptable for all of the pesticides, but significantly superior to that of state-of-the-art packed column gas chromatography. During the course of the study, it was observed that relative to other pesticides, the response factor (area/ng) for endrin progressively decreased while those of the decomposition products, endrin aldehyde and endrin ketone, increased. Varying the injector temperatures between 150°C and 250°C had no effect on the apparent decomposition of endrin. Successive analyses of a freshly prepared standard solution containing only reagent grade endrin also resulted in a chromatogram of symmetrical peaks with the correct retention times for endrin, endrin aldehyde, and endrin ketone indicating that the original standard was stable. Packed column analyses using a mass spectrometer as a detector of the same

standard confirmed the presence of only endrin. The capillary column was subsequently replaced with one of the same kind. However, no improvement in the system performance was observed for endrin. To this point, only standard solutions were analyzed (mixtures of priority pollutants only in hexane). The injector sleeve was inspected and a dark area was observed at the point of injection. The sleeve was washed with detergent solution followed by distilled water, acetone, and hexane rinsings. The sleeve was reinstalled, conditioned, and retested. No improvement in performance was observed for endrin. The sleeve was again removed, washed, dried, then silane treated by soaking in a toluene solution of dimethyldichlorosilane for 10 minutes. An immediate improvement in the system performance to endrin was noted. However, after a few days, a significant reduction in the response factor to endrin was again noted. The injector liner was again removed and deactivated by chemically bonding Carbowax 20M to its surface. In accordance with the method of Aue[7], the liner was soaked in a 5% solution of Carbowax 20M in methylene chloride for five minutes, allowed to air-dry, then placed in a tube oven at room temperature. Oxygen-free helium was allowed to flush the oven for 10 minutes, then the oven was slowly heated to 280°C. This temperature was maintained for 18 hours with a helium flow. The oven was cooled to room temperature, then the liner was reinstalled in the cool capillary system injector housing. After a proper conditioning period, subsequent injections of endrin indicated no evidence (< 1%) of peaks for endrin aldehyde and endrin ketone present in the chromatograms. After one week of stable operation, endrin response factors slowly started to decrease with the appearance of endrin aldehyde and endrin ketone. At the end of three weeks of operation, significant (> 50%) endrin breakdown was again evident. No other sleeve liner deactivation studies such as the use of other immobilized phases or persilylation has been investigated.

Column Life

The initial system performance tests were performed using a 30m x 0.32 mm I.D., 0.25 µm df, column coated with SE-54. Standards and extracts of pesticide spiked reagent water were routinely injected into the instrument. Each sample extract and reference standard solution was spiked with an internal standard solution at the rate of 30 ng/mL 4,4'-dibromobiphenyl, 10 ng/mL decafluorobiphenyl and 10 ng/mL 4,4'-dibromooctafluorobiphenyl. These early eluting internal standards were used to monitor injection technique and, to compare relative retention time data using the GC data system. Since these compounds do not elute in areas of the analytes, they are not suitable as quantitative internal standards. After about four weeks of column operation, the peak geometry for 4,4'-dibromobiphenyl was observed to degrade, resulting in an abnormally wide severely tailing peak. All other internal standard and priority pollutant peaks appeared normal. Unfortunately, since an electron capture detector was used, a check on the capillary system using a Grob polarity test mixture could not be performed. Approximately one week later, the peak geometry for the organochlorine pesticides began to degrade in a similar fashion. The column was replaced with a column coated with OV-17, returning the system to its original inert condition. Again, the same succession of events occurred over a similar period of time. First, the peak geometry of 4,4'-dibromobiphenyl degraded, followed by the organochlorine pesticides. Neither column could be regenerated by discarding the first 5 m of the column as recommended by the manufacturers. At this point, bonded liquid phase columns became commercially available. The column was replaced with a polymethyl (5% phenyl) siloxane bonded phase column with a 0.25 µm df. Extracts from natural waters, drinking water, sediments, and process streams

were routinely injected into this column over a period of 18 months before any adverse effects were noted. As with the non-bonded phase columns, the peak geometry for 4,4'-dibromobiphenyl degraded before other analytes were affected. No attempt was made to regenerate the column by solvent flushing because the polyimide exterior coating on the fused silica became brittle with age causing the column to break into several sections as it was removed from the instrument.

Injector Study

For the 600-series liquid/liquid extraction methods, methylene chloride is used to extract priority pollutants from various aqueous matrices. It is not a desirable solvent for column clean-up operations and non mass spectrometer chromatographic injections because of its polarity and volatility, and because of the adverse effects it has upon electron capture and nitrogen-phosphorus detectors. For those methods in the 600-series which require the use of these options, methylene chloride is exchanged to hexane (mixed hexanes). A quantity of mixed hexanes is added to the sample extract. During the evaporative concentration step, the methylene chloride and the most volatile hexanes are evaporated, leaving the sample dissolved in the higher boiling hexane fractions. The boiling point of the resulting mixture varies, depending upon the organic content of the sample, the quantity and boiling range of the added hexane, and the final volume selected. Other extraction procedures (2) use a mixture of hexane and methylene chloride as the extracting solvent, further complicating this problem because of the relatively large volume of hexane used. For packed column gas chromatography, analysts found that if the standard is prepared in the same solvent used in

the final concentration step, sample/standard area discrepancies due to slight boiling point differences are minimal and within the experimental error of the method. To determine what effect the solvent boiling point variances may have upon splitless capillary injection, three complex standard mixtures were prepared in pentane BP 35-37°C, mixed hexanes BP 68-69°C, and 2,2,4-trimethylpentane (isooctane) BP 99°C. The analytes and their concentrations are listed in Table 2. The gas chromatograph was set up according to the manufacturer's recommended operating conditions. Using automatic splitless injections, approximately 1.8 µL of sample was injected into a 2.0 x 79 mm glass injector sleeve operated at 220°C. Just prior to needle penetration, the injector flush flow was turned off. Thirty seconds after injection, the injector flush flow was reestablished. It should be noted that the autosampler as supplied by the manufacturer injected samples under conditions not ideal for splitless injections,[8], that is,

(1) The needle was filled with sample during septum penetration. (Ideal: The needle should be free of sample and allowed to heat up before injection.)

(2) The sample was rapidly injected. (Ideal: The sample should be injected at a slow rate (i.e. 1 µL/second) so that the sleeve liner does not flood with the volatilized sample.

(3) The syringe was withdrawn immediately after injection. (Ideal: The syringe should remain in the injector 10 to 15 seconds to allow high boilers to volatilize.)

The following multi-ramp temperature program was selected which resolves all the priority pollutant pesticides. The 30M x 0.32mm ID, 0.25 µm df

TABLE 2

Organochlorine Pesticides

Compound	Concentration[a] ng/μL
α-BHC	0.01
β-BHC	0.01
γ-BHC	0.01
Δ-BHC	0.01
Heptachlor	0.005
Aldrin	0.008
Heptachlor epoxide	0.008
γ-Chlordane	0.01
Endosulfan I	0.01
P,P'-DDE	0.01
Dieldrin	0.01
Endrin	0.02
Endosulfan II	0.01
P,P'-DDD	0.01
Endrin aldehyde	0.02
Endosulfan sulfate	0.04
P,P'-DDT	0.04
Endrin ketone	Not present
Methoxychlor	0.06
Mirex	0.01

[a]Concentration of analyte in pentane, hexane, 2,2,4-Trimethylpentane and "Samples."

polymethyl (5% phenyl) siloxane bonded phase column was maintained at 90°C for five minutes then rapidly programmed at 12°/minute to 230°C. At 230°C, the program rate was changed to 5°/minute until the column temperature reached 250°C. The column was maintained at 250°C for 21 minutes, then recycled to 90°C for the next analysis. Retention data for each of the pesticides using this program are discussed under Retention Data Studies. When splitless injections were made using samples diluted in pentane or hexane sample, reconstitution was due to cold trapping only. For samples dissolved in isooctane, the initial column temperature was proper for reconstitution by the "solvent effect" assuming that sufficient solvent volume was injected. No effort was made to determine what minimum injection volumes were required for solvent effect reconstitution.

The chromatograms, peak areas, RSD of peak areas, peak heights, peak widths, and retention data of the resulting injections were then compared. It was found that peak widths and retention data were the same for the three mixtures; however, severe differences were noted between the areas for each analyte as the solvent was changed. Altering injection time parameters did not improve the agreement. From the results of this test, it was apparent that if the samples are not contained in exactly the same solvent, then significant errors will result if external standard quantitative measurements are performed. Since identical standard/sample solvents are not within the scope of current environmental methodology, additional investigations were conducted in an effort to minimize this error. The problem was discussed with the manufacturer factory personnel. It was theorized that as the sample is injected into the sleeve, the low boiling solvents volatilize in an explosive manner causing the injector sleeve to flood, spilling a significant portion of

the sample out of the top of the injector into the injector system's pneumatic vents. It was recommended that a smaller sample volume be injected and a larger volume injector sleeve be used.[9] The experiment was duplicated using a special 2.1 mm I.D. sleeve. The auto injector was adjusted to deliver a minimal solvent volume (about 0.5 µL + 0.8 µL needle volume). The result was even poorer agreement between replicate injections of the three different solvents. Since the opposite effect was obtained, the injection volume was increased to 2 µL (plus the 0.8 µL needle volume). Replicate injections were again analyzed from each of the three solvents. The area agreement between the solvents improved, however, they were still far from acceptable for quantitative measurements. Table 3 lists the average areas for six analyses and the relative standard deviations (RSD) for each of the compounds tested. It is apparent that with the exception of endrin and endrin aldehyde, the RSD between replicate injections for a given solvent is acceptable; however, the peak area agreement between various apolar solvents is not acceptable for external standard quantitative measurements. (The endrin and endrin aldehyde demonstrated poor RSD because the sleeve liner was not deactivited for this study.)

For packed column gas chromatography, the quality of quantitative data resulting from imprecise injection practices can usually be improved by incorporating internal standard calibrations. Since the area variances shown in Table 3 are injector related and appear to be uniformly predictable, it was decided to reexamine the data using internal standards techniques. Ideally, internal standards should be chemically and physically similar to the analytes and elute in an adjacent interference-free area of the chromatogram. The halogenated biphenyls previously mentioned as qualitative internal standards

TABLE 3

Area Measurement
Automatic Injection
Empty Injector Sleeve

Compound	Average Area (RSD)[a]		
	Pentane	Hexane	Isooctane
α-BHC	188.2 (8.3)	440.2 (5.1)	703.2 (3.1)
β-BHC	130.4 (8.3)	319.6 (5.5)	479.7 (2.7)
γ-BHC	117.4 (8.4)	307.3 (6.4)	493.4 (3.4)
Δ-BHC	124.6 (8.3)	307.3 (6.4)	493.4 (3.4)
Heptachlor	192.3 (15.2)	490.3 (4.8)	731.9 (4.0)
Aldrin	217.1 (8.5)	479.8 (4.7)	698.8 (2.1)
Heptachlor epoxide	256.5 (9.8)	599.4 (5.4)	866.7 (3.4)
γ-Chlordane	118.5 (5.0)	261.0 (7.7)	377.0 (4.5)
Endosulfan I	305.9 (9.0)	728.2 (5.9)	1056 (2.8)
P,P'-DDE	280.1 (8.3)	656.5 (5.3)	945.3 (2.7)
Dieldrin	246.9 (8.0)	575.9 (5.4)	828.1 (3.2)
Endrin	68.8[b]	216.6 (4.1)	491.1 (17.6)
Endosulfan II	273.2 (6.8)	657.2 (5.3)	971.4 (3.4)
P,P'-DDD	149.4 (7.2)	341.5 (5.0)	489.1 (2.8)
Endrin aldehyde	637.6 (6.8)	1498 (4.8)	2011 (5.1)

TABLE 3. Continued

Compound	Average Area (RSD)[a]		
	Pentane	Hexane	Isooctane
Endosulfan sulfate	963.7 (7.2)	2399 (5.0)	3687 (4.6)
P,P'-DDT	651.6 (12.8)	1810 (6.2)	2762 (3.1)
Endrin ketone	131.0 (9.4)	324.2 (5.2)	434.3 (4.6)
Methoxychlor	395.2 (14.4)	1274 (4.9)	2068 (5.6)
Mirex			

a Average area (integrator counts) and relative standard deviation (%) for 6 replicate injections using an autosampler.

b Only one data point.

were tested and found unsuitable for this purpose. In lieu of selecting additional internal standards and reanalyzing the samples, endosulfan-I was selected as an internal standard because it is a mid-range eluter and consistently has a low area RSD for replicate injections. Using the data listed in Table 3, the average area response for endosulfan-I was divided into the average area response for each of the remaining pesticides. The resulting area ratios are listed in Table 4. This table clearly shows that within experimental error, the area ratio for those pesticides eluting near endosulfan-I is constant as the solvent is changed. Endrin, endrin aldehyde and endrin ketone were not included in this observation due to variable endrin breakdown in the injector.

Further modifications were then performed upon the injector system in an effort to improve sample introduction into the column. The first 1 cm of the 2.1 x 79 mm sleeve liner was packed with silane treated glass wool. Replicate injections from each of the three standards were again analyzed. Improvement in analyte area agreement was apparent as the solvent system was varied, however, the agreement was still not sufficient for external calibration. The liner was then packed with long-filament, silane treated glass wool to form a continuous plug extending 3.5 cm down the injector sleeve. Measurements indicated that the syringe needle of the auto injector would penetrate the glass wool and expose about 1 mm of the tip below the glass wool plug during injection. Several test injections were made to verify that needle penetration would not displace the glass wool plug and that the needle actually completely penetrated the glass wool plug prior to injection. The experiment was again repeated using multiple automated injections of the standards contained in the three solvents. Table 5 lists the resulting data.

TABLE 4

Area Ratio
Automatic Injection
Empty Injector Sleeve

Compound	Area Ratio[a]		
	Pentane	Hexane	Isooctane
α-BHC	0.615	0.605	0.666
β-BHC	0.427	0.439	0.454
γ-BHC	0.384	0.393	0.391
Δ-BHC	0.407	0.422	0.467
Heptachlor	0.629	0.673	0.693
Aldrin	0.708	0.659	0.662
Heptachlor epoxide	0.839	0.823	0.821
γ-Chlordane	0.378	0.358	0.357
Endosulfan I	1.000	1.000	1.000
P,P'-DDE	0.916	0.902	0.895
Dieldrin	0.807	0.791	0.784
Endrin	0.225	0.297	0.465
Endosulfan II	0.893	0.902	0.920
P,P'-DDD	0.488	0.469	0.463
Endrin aldehyde	2.084	2.057	1.904
Endosulfan sulfate	3.150	3.294	3.492
P,P'-DDT	2.130	2.486	2.616
Endrin ketone	0.428	0.445	0.411
Methoxychlor	1.291	1.750	1.958
Mirex	1.638	1.449	1.377

a Area ratio = $\frac{\text{Average area analyte}}{\text{Average Area Endosulfan-I}}$

TABLE 5

Area Measurement and Area Ratio
Automatic Injection
Packed Injector Sleeve

	Ave. Area(RSD)[a]			Area Ratio[b]		
	Pentane	Hexane	Isooctane	Pentane	Hexane	Isooctane
α-BHC	1466 (23.7)	1270 (3.4)	1244 (3.9)	0.712	0.651	0.630
β-BHC	1005 (20.0)	882.5 (3.1)	861.5 (2.8)	0.488	0.452	0.436
γ-BHC	816.4 (21.0)	711.6 (2.9)	696.2 (2.9)	0.396	0.365	0.353
Δ-BHC	995 (22.3)	851.8 (4.0)	743.9 (3.9)	0.483	0.437	0.377
Heptachlor	1235 (16)	1105 (3.1)	1104 (2.6)	0.600	0.567	0.559
Aldrin	1520 (18.9)	1399 (2.4)	1380 (1.9)	0.738	0.717	0.699
Heptachlor epoxide	1686 (19.3)	1521 (2.5)	1586 (2.0)	0.818	0.780	0.803
γ-Chlordane[c]		623.1 (4.5)			0.320	
Endosulfan I	2050 (19.9)	1950 (2.2)	1975 (1.9)	1.000	1.000	1.000
P,P'-DDE	2005 (20.0)	1961 (1.5)	2035 (1.6)	0.973	1.006	1.030
Dieldrin	1673 (19.1)	1622 (1.7)	1646 (2.8)	0.812	0.832	0.833
Endrin	983.2 (11.2)	1119 (9.4)	1043 (11.2)	0.477	0.574	0.528
Endosulfan II	1833 (18.6)	1836 (1.4)	1901 (3.4)	0.890	0.942	0.963
P,P'-DDD	1093 (18.2)	1059 (0.9)	1089 (2.3)	0.531	0.543	0.551

TABLE 5. Continued

	Ave. Area(RSD)[a]			Area Ratio[b]		
	Pentane	Hexane	Isooctane	Pentane	Hexane	Isooctane
Endrin aldehyde	3626 (15.7)	3342 (2.8)	3259 (4.0)	1.760	1.714	1.650
Endosulfan sulfate	6443 (19.3)	6405 (2.2)	6645 (5.0)	3.128	3.285	3.365
P,P'-DDT	5168 (22.0)	5253 (5.4)	5445 (3.6)	2.509	2.694	2.757
Endrin	662.3 (17.9)	625.1 (1.2)	644.3 (6.3)	0.321	0.321	0.326
Methoxychlor	4044 (18.1)	4263 (5.1)	4665 (5.7)	1.963	2.186	2.362
Mirex	2220 (17.3)	2251 (5.9)	2350 (3.1)	1.078	1.162	1.190

[a] Average area (integrator counts) and (relative standard deviation) for 6 replicate injections using an autosampler.

[b] $\text{Area Ratio} = \frac{\text{Ave. area analyte}}{\text{Ave. area Endosulfan I}}$

[c] No data due to interference.

Excellent agreement was achieved between the average areas for the hexane and the isooctane solvent standards. The average area value for the pentane solvent standard appears to agree well with the results obtained for the other two solvents. However, the RSD between pentane injections is excessive when compared to previous replicate injections (Table 4). As a result, the experiment was repeated using a less complex sample. Again, average area measurement agreement between solvents was acceptable, but a high RSD was encountered with replicate pentane injections. The higher RSD between replicate injections of pentane is also apparent to a lesser degree in Table 3. For a given injection, the relative imprecision of pentane injections appears to be independent of the analytes. Therefore, it is likely that the problem is associated with the high volatility of the sample solvent and the inability of the autosampler to deliver a constant volume of such solvents into the injector. The poor RSD obtained from samples dissolved in pentane indicates that this is an unsuitable solvent for environmental samples if automated splitless injection techniques are used. For other environmental methods where the final extract is contained in methylene chloride (BP 40-41^{o}C), this also could be a problem and should be taken into consideration if totally automated splitless injections are included into the analysis scheme. As a result of these experiments, it appears that if external calibration techniques are to be used for the splitless injection of environmental extracts, modifications to the liner may be required to contain and deliver the sample to the column in a consistent and predictable manner. As in the case of Table 4, the area ratio data in Table 5 appears independent of the solvent indicating that internal standard calibration techniques could also be used for glass wool packed liner injections.

As these data were being collected with the automatic injector, manual injections were also performed using the unpacked injector sleeve and the 3.5 cm glass wool packed sleeve. Solvent flush injections were made where a 10 µL syringe was loaded with 0.8 µL (needle volume) of clean solvent followed by 1.0 µL of air and finally 3 µL of the sample. "Hot needle" injection techniques were used where the tailing edge of the sample was drawn back into the syringe to the 1 µL mark. The needle was fully inserted into the injector while the injector flows were operated in the "flush" mode for 10 seconds. Then the injector flush flow was turned off and the sample was injected at the rate of 1 µL/second. After 30 seconds, the flush flow was reestablished and the needle withdrawn from the septum. Table 6 lists the results of duplicate and triplicate manual injections into the 2.1 x 79 mm injector sleeve. The area responses were divided by the volume of sample injected (typically 3 µL) and averaged. As in the case of the automatic injections, there are significant variations in the analyte area as the solvent is changed.

Table 7 lists the results of replicate manual injections into a 2.1 x 79 mm packed with 3.5 cm of glass wool. The area responses were again divided by the volume injected (3 µL) and averaged. Because of the random nature of these data, it is difficult to determine from these data if packing the injector sleeve with glass wool improves the agreement between solvents for manual injections. Since automatic injections had clearly demonstrated that precise data can be obtained from splitless injections, an effort was made to identify the manual injection problem and improve upon the precision between replicate injections. Table 8 shows the effect of varying the injecting volumes of sample dissolved in isooctane from one to six µL. In this Table the area responses were divided by the volume injected. Ideally the resulting

TABLE 6

Area Measurement and Area Ratio
Manual Injection
Empty Injector Sleeve

	Average Area (RSD)[a]			Average of Area Ratio (RSD)[b]		
	Pentane[c]	Hexane[d]	Isooctane[e]	Pentane[c]	Hexane[d]	Isooctane[e]
α-BHC	94.3 (1.2)	104.0 (9.5)	147.3 (5.2)	0.659 (2.2)	0.583 (4.9)	0.656 (9.3)
β-BHC	69.0 (2.8)	91.3 (17)	133.3 (5.3)	0.482 (3.2)	0.535 (4.6)	0.594 (11.)
γ-BHC	58.5 (5.2)	74.6 (5.6)	97.7 (6.0)	0.480 (6.1)	0.420 (3.2)	0.440 (7.7)
Δ-BHC	50.8 (1.8)	72.5 (17)	105.4 (9.9)	0.354 (2.8)	0.406 (8.7)	0.467 (6.5)
Heptachlor	104.0 (3.8)	84.3 (5.0)	119.0 (24)	0.722 (4.5)	0.477 (14)	0.520 (12)
Aldrin	112.0 (2.7)	131.0 (3.8)	169.3 (5.0)	0.781 (3.5)	0.734 (4.6)	0.755 (9.9)
Heptachlor Epoxide	122.0 (0.7)	168.0 (14)	228.3 (6.2)	0.848 (1.3)	0.940 (5.4)	1.014 (8.5)
γ-Chlordane	54.5 (0.1)	80.3 (16)	110.7 (4.6)	0.380 (0.7)	0.450 (7.7)	0.496 (16)
Endosulfan I	143.0 (0.5)	178.0 (8.7)	226.7 (13)	1.000	1.000	1.000
P,P'-DDE	136.0 (1.0)	212.0 (17)	284.7 (2.7)	0.949 (0.5)	1.189 (8.2)	1.272 (14)
Dieldrin	110 (1.9)	101 (0)	187.7 (18)	0.766 (0.9)	0.522 (8.7)	0.826 (4.6)
Endrin	149 (6.2)	100 (14)	227 (35)	1.032 (5.6)	0.571 (22)	0.982 (24)
Endosulfan II	118.0 (1.8)	71.7 (3.3)	226.0 (36)	0.820 (2.7)	0.405 (12)	0.846 (7.9)
P,P'-DDD	76.3 (2.2)	93.3 (18)	167.3 (0.4)	0.532 (1.6)	0.523 (8.5)	0.748 (14)

TABLE 6. Continued

	Average Area (RSD)[a]			Average of Area Ratio (RSD)[b]		
	Pentane[c]	Hexane[d]	Isooctane[e]	Pentane[c]	Hexane[d]	Isooctane[e]
Endrin aldehyde	204 (6.6)	104 (6.1)	184.7 (21)	1.421 (7.2)	0.590 (14)	0.809 (9.3)
Endosulfan sulfate	384 (1.7)	218 (4.2)	646.3 (19)	2.675 (1.7)	1.231 (13)	2.839 (6.8)
P,P'-DDT	291 (5.1)	258 (12)	498.7 (14)	2.024 (4.3)	1.443 (3.1)	2.199 (2.1)
Endrin ketone	37.9 (7.1)	36.1 (0.8)	55.2 (2.8)	0.265 (7.8)	0.240 (9.7)	0.247 (16)
Methoxychlor	242 (6.7)	142 (1.5)	486 (16)	1.685 (6.1)	0.799 (10.0)	2.142 (4.8)
Mirex	202 (1.8)	261 (20)	386 (2)	1.406 (1.2)	1.459 (11)	1.726 (16)

[a] Average of $\frac{\text{Area}}{\text{Injection Volume } (\mu L)}$; (relative standard deviation)

[b] Average of $\frac{\text{Area of Analyte}}{\text{Area Endosulfan I}}$; (and relative standard deviation)

[c] Number of Runs = 2
[d] Number of Runs = 2
[e] Number of Runs = 3

TABLE 7

Area Measurement and Area Ratio
Manual Injection
Packed Injector Sleeve

	Average Area (RSD)[a]			Average of Area Ratio (RSD)[b]		
	Pentane[c]	Hexane[d]	Isooctane[e]	Pentane[c]	Hexane[d]	Isooctane[e]
α-BHC	117 (4.3)	109 (23)	158 (9.1)	0.549 (3.4)	0.519 (27)	0.517 (8.1)
β-BHC	98.0 (3.0)	104 (9.4)	148 (7.2)	0.462 (2.8)	0.490 (10)	0.484 (4.6)
γ-BHC	76.7 (4.9)	72.6 (15)	105 (8.0)	0.436 (21)	0.345 (19)	0.343 (6.2)
Δ-BHC	86.7 (2.0)	93.2 (15)	142 (13)	0.408 (2.8)	0.439 (9.1)	0.471 (8.1)
Heptachlor	130 (1.6)	97.4 (36)	143 (9.3)	0.611 (1.0)	0.469 (43)	0.468 (8.5)
Aldrin	147 (4.8)	157 (4.7)	229 (7.0)	0.691 (4.0)	0.738 (8.0)	0.747 (2.1)
Heptachlor epoxide	181 (2.0)	200 (12)	287 (6.7)	0.847 (1.3)	0.935 (5.0)	0.936 (2.6)
γ-Chlordane	75.4 (3.1)	80.1 (9.6)	123 (9.9)	0.354 (2.6)	0.375 (4.0)	0.402 (6.1)
Endosulfan I	213 (1.0)	221 (13)	307 (4.9)	1.000	1.000	1.000
P,P'-DDE	212 (2.7)	241 (19)	353 (8.5)	0.997 (1.7)	1.122 (14)	1.149 (4.2)
Dieldrin	179 (1.6)	153 (13)	228 (7.2)	0.843 (2.4)	0.719 (17)	0.747 (7.4)
Endrin	258 (2.5)	140 (39)	165 (40)	1.215 (2.0)	0.677 (46)	0.542 (42)
Endosulfan II	198 (5.3)	153 (25)	250 (13)	0.934 (6.4)	0.724 (28)	0.817 (13)
P,P'-DDD	123 (1.1)	137 (23)	188 (8.1)	0.584 (0.4)	0.636 (17)	0.613 (3.9)

TABLE 7. Continued

	Average Area (RSD)[a]			Average of Area Ratio (RSD)[b]		
	Pentane[c]	Hexane[d]	Isooctane[e]	Pentane[c]	Hexane[d]	Isooctane[e]
Endrin aldehyde	319 (13)	254 (30)	392 (30)	1.501 (14)	1.205 (33)	1.453 (6.8)
Endosulfan sulfate	679 (9.9)	496 (30)	898 (4.7)	3.173 (11)	2.339 (32)	2.935 (6.4)
P,P'-DDT	507 (2.7)	389 (11)	615 (9.8)	2.388 (3.4)	1.826 (9.6)	2.009 (9.2)
Endrin ketone	38.1[f]	57.1 (26)	105 (20)	0.180[f]	0.265 (20)	0.341 (18)
Methoxychlor	499 (4.5)	329 (34)	623 (8.0)	2.350 (5.3)	1.550 (34)	2.038 (11)
Mirex	306 (2.1)	307 (22)	418 (7.4)	1.439 (1.3)	1.429 (16)	1.365 (3.6)

[a] Average of $\frac{\text{Area}}{\text{injection volume } (\mu L)}$; (and relative standard deviation)

[b] Average of $\frac{\text{Area analyte}}{\text{Area Endosulfan-I}}$; (and relative standard deviation)

[c] Number of Runs = 2
[d] Number of Runs = 4
[e] Number of Runs = 4
[f] 1 data point

TABLE 8

Manual Injection - Effect of Injection Volume

	Response[a] and (Response Factor)[b]					
	1.0µL	2.0µL	2.9µL	3.95µL	4.95µL	6.0µL
α-BHC	153 (0.810)	113 (0.549)	194 (1.032)	179 (0.932)	198 (0.664)	384 (1.150)
β-BHC	134 (0.709)	114 (0.553)	172 (0.915)	164 (0.854)	157 (0.527)	264 (0.790)
γ-BHC	99.2 (0.525)	78.8 (0.383)	124 (0.660)	116 (0.604)	120 (0.403)	203 (0.608)
Δ-BHC	86.8 (0.459)	93.5 (0.454)	152 (0.809)	163 (0.849)	158 (0.530)	257 (0.769)
Heptachlor	105 (0.556)	92.7 (0.450)	84.4 (0.449)	64.4 (0.335)	170 (0.570)	135 (0.404)
Aldrin	213 (1.127)	170 (0.825)	266 (1.415)	249 (1.297)	238 (0.799)	388 (1.162)
Heptachlor epoxide	190 (1.005)	186 (0.903)	228 (1.213)	242 (1.260)	277 (0.930)	406 (1.216)
γ-Chlordane	84.6 (0.448)	82.5 (0.400)	171 (0.910)	149 (0.776)	118 (0.396)	195 (0.584)
Endosulfan I	189 (1.000)	206 (1.000)	188 (1.000)	192 (1.000)	298 (1.000)	334 (1.000)
P,P'-DDE	352 (1.862)	296 (1.437)	482 (2.564)	450 (2.344)	340 (1.141)	657 (1.967)
Dieldrin	171 (0.905)	174 (0.845)	168 (0.894)	174 (0.906)	234 (0.785)	148 (0.443)
Endrin	152 (0.804)	178 (0.864)	86.1 (0.458)	89.5 (0.466)	238 (0.799)	94 (0.281)
Endosulfan II	137 (0.725)	171 (0.830)	172 (0.915)	210 (1.094)	254 (0.852)	170 (0.509)
P,P'-DDD	134 (0.709)	147 (0.714)	148 (0.787)	153 (0.797)	167 (0.560)	254 (0.760)
Endrin aldehyde	177 (0.937)	216 (1.049)	233 (1.239)	285 (1.484)	400 (1.342)	199 (0.596)

TABLE 8. Continued

	Response[a] and (Response Factor)[b]					
	1.0 µL	2.0µL	2.9µL	3.95µL	4.95µL	6.0µL
Endosulfan sulfate	345 (1.825)	480 (2.330)	496 (2.638)	647 (3.370)	939 (3.151)	690 (2.066)
P,P'-DDT	330 (1.746)	372 (1.806)	329 (1.750)	309 (1.609)	688 (3.209)	704 (2.108)
Endrin ketone	-	60.6 (0.294)	99.6 (0.530)	113 (.589)	84.2 (0.283)	84.5 (0.253)
Methoxychlor	289 (1.529)	384 (1.864)	356 (1.894)	400 (2.083)	698 2.342	437 (1.308)
Mirex	378 (2.000)	337 (1.636)	478 (2.543)	404 (2.104)	387 (1.299)	656 (1.964)

a $\frac{\text{Area}}{\text{injection volume (µL)}}$

b Response Factor = $\frac{\text{Area analyte}}{\text{Area Endosulfan-I}}$

value should be constant for a given analyte. The values appear to be random over the entire injection volume range studied. Based upon the data gathered in Table 7 and 8 it is evident that commonly used manual injection techniques can generate totally unacceptable quantitative data over the entire sample volume injection range normally associated with splitless injections. Other attempts to resolve the problem by carefully duplicating all operations, using "cold needle" injections, rapid injections, new syringes did not help. Ultimately, it was found that by bending the last cm of the syringe needle approximately 1.5 mm, so that the needle tip always contacted the injector sleeve during injection, significantly improved the precision between replicate injections. However, this practice does not properly resolve the problem because the injector septum fails after only a few injections. From this limited experimental work, it appears that the poor precision obtained from manual injections is related primarily with the needle location within the oversized injector sleeve chosen for this study. Internal standard (area ratios) shown in Tables 6, 7, and 8 also reflect unacceptably high RSD, and in the case of Table 8, poor agreement between injection volumes substantiating that the variance is due to inability of this injector design to, volatilize, and deliver to the column a predictable amount of sample during manual injections.

Automated Splitless Injection of Concentrated Solvents

In order to determine what magnitude of error concentrated solvents may have upon Method 608 and the pesticide method used for NIPDWR[2] when automated splitless capillary injections are performed, the following experiments were performed. For Method 608, 180 mL of methylene chloride was concentrated by

Kuderna-Danish (K-D) evaporative concentration techniques down to approximately 6 mL. Fifty mL of hexane (boiling range 68-69°C) was added to the reservoir and the mixture was again concentrated down to approximately 6 mL. Once cool, the mixture was transferred to a 10.0 mL volumetric flask spiked with pesticides then diluted to volume with hexane. The concentration of each pesticide in the resulting mixture is listed in Table 2. This standard solution of pesticides contained in K-D concentrated solvents closely approximates the conditions associated in the extraction/concentration procedures (assuming 100% extraction efficiency). For simplicity in this section such mixtures hereafter will be referred to as "samples." Three "samples" were prepared in this manner and each "sample" was analyzed in duplicate. For the pesticide method required in NIPDWR[2], 180 mL of hexane (boiling range 68-69°C) was concentrated by K-D to approximately 6 mL (ethyl ether normally included in the extraction solvent was not included). Once cool, the mixture was transferred to a 10.0 mL volumetric flask spiked with pesticides and diluted to volume with hexane. The resulting concentration of each pesticide is shown in Table 2. Four "samples" were prepared in this manner and each was analyzed in duplicate. Four separate 10 mL standards were prepared at the same levels shown in Table 2 by spiking hexane (boiling range 68-69°C). Each standard was analyzed in duplicate. The "samples" and standards were randomly analyzed over a 15 hours. These two studies were evaluated using a "t" test at the 95% confidence level. The results are shown in Tables 9 and 10.

The "t" values shown in these tables are a comparison of each experimental value also taking into account the standard deviations of the standards and

TABLE 9

SPLITLESS BIAS FOR SAMPLES
EXTRACTED WITH METHYLENE CHLORIDE

	Area[a]	Standard[b] Deviation	Area[c]	Standard[d] Deviation	Sample t[e]	Sample Bias[f] (Percent)
α-BHC	471	19.0	440	7.3	3.5	93
β-BHC	303	8.7	285	7.8	3.5	94
δ-BHC	268	7.8	255	4.5	3.4	95
Δ-BHC	289	8.4	273	8.7	2.9	95
heptachlor	498	13.0	467	8.4	4.2	94
aldrin	499	18.0	475	8.9	0.8	95
heptachlor epoxide	570	18.0	556	16.0	1.5	98
δ-chlordane	249	21.0	229	6.7	2.4	92
Endosulfan-I	649	25.0	623	20.0	1.8	96
p,p'DDE	583	31.0	571	22.0	0.7	98
Dieldrin	527	20.0	519	17.0	0.7	98
Endrin	745	135.0	523	41.0	6.9	70
Endosulfan-II	563	27.0	542	22.0	1.2	96
p,p'-DDD	300	22.0	297	11.0	1.8	99
Endrin aldehyde	964	87.0	961	42.0	-0.5	100
Endosulfan sulfate	2150	113.0	2030	93.0	1.5	94
p,p'-DDT	1610	105.0	1550	73.0	1.2	96
Methoxychlor	1290	97.0	1190	68.0	2.2	92
Mirex	882	54.0	841	34.0	1.5	95
					Bias Ave[g]	95

a Average area of standard
b Standard deviation of 6 standards
c Average area of sample
d Standard deviation of 6 samples
e students "t" test value where a t value larger than 2.6 at the 95% confidence level is statistically significant
f Bias = ave. $\frac{\text{area sample}}{\text{area standard}}$ X 100
g Average Bias not including data for Endrin and Endrin aldehyde

TABLE 10

SPLITLESS BIAS FOR SAMPLES
EXTRACTED WITH NARROW BOILING RANGE HEXANE

	Standard				Sample	
	Area[a]	Standard Deviation[b]	Area[c]	Standard Deviation[d]	t[e]	Bias[f] (Percent)
α-BHC	471	19.0	437	29	2.4	93
β-BHC	303	8.7	283	19	2.6	93
δ-BHC	268	7.8	252	16	2.4	94
Δ-BHC	289	8.4	264	18	4.1	91
heptachlor	498	13.0	476	26	2.1	96
aldrin	499	18.0	475	30	2.0	95
heptachlor epoxide	570	18.0	542	34	2.9	95
δ-chlordane	249	21.0	227	13	2.2	91
Endosulfan-I	649	25.0	617	37	3.4	95
p,p'DDE	583	31.0	562	34	3.6	96
Dieldrin	527	20.0	501	25	5.1	95
Endrin	745	135.0	671	118	3.1	90
Endosulfan-II	563	27.0	529	24	5.1	94
p,p'-DDD	300	22.0	268	13	4.4	89
Endrin aldehyde	964	87.0	808	112	4.6	89
Endosulfan sulfate	2150	113.0	1970	78	7.6	92
p,p'-DDT	1610	105.0	1540	72	3.7	96
Methoxychlor	1290	97.0	1230	64	3.1	95
Mirex	882	54.0	830	34	4.6	94
					Bias Ave[g]	94%

a Average area of standard
b Standard deviation of 8 standards
c Average area of sample
d Standard deviation of 8 samples
e Students "t" value where a t value larger than 2.4 at the 95% confidence level is statistically significant
f Bias = $\text{ave. } \frac{\text{area sample}}{\text{area standard}} \times 100$
g Average bias not including data for endrin and endrin aldehyde

the "samples." For six data points at the 95% confidence level, a "t" value larger than 2.6 indicates a statistically significant difference; for eight data points, a "t" value larger than 2.4 is significant. A comparison of the "t" values listed in these tables shows that for the majority of the analytes there is a slight but statistically significant difference between the standards and the "samples." It is curious to note that the "samples" show a consistant negative bias for both methods. This is unexpected since the anticipated effects of K-D concentration should result in a positive bias. When compared to other methodology errors normally associated with liquid-liquid extraction procedures on diverse sample types, this slight negative analytical bias appears to be insignificant. The experiment was repeated again; this time a wide boiling range solvent was selected and concentrated according to the NIPDWR[2] method. One hundred and eighty mL of petroleum ether (boiling range 30-60°C) was concentrated by K-D to approximately 6 mL. The mixture was transferred to a 10.0 mL volumetric flask spiked with pesticides and diluted to 10.0 mL using petroleum ether (boiling range 30-60°C) at concentrations shown in Table 2. Three "samples" were prepared in this manner. Three 10 mL standards at the concentrations shown in Table 2 were also prepared using petroleum ether (boiling range 30-60°C) as the solvent. The three standards and "samples" were randomly analyzed in duplicate. The results of this study are shown in Table 11. It is obvious from these data that there is a significantly different response of the chromatographic system to identical concentrations of each analyte contained in the standard and "sample." The net result is if such a solvent were substituted for a narrow boiling range extracting solvent, the the analytical step could account for as much as a +23% bias. The reported average single

TABLE 11

SPLITLESS BIAS FOR SAMPLES
EXTRACTED WITH WIDE BOILING RANGE SOLVENT

	Standard				Sample	
	Area[a]	Standard Deviation[b]	Area[c]	Standard Deviation[d]	t[e]	Bias[f] (Percent)
α-BHC	345	17.1	421	4.0	-12.5	122
β-BHC	255	11.4	312	5.9	-12.6	122
δ-BHC	217	10.4	263	2.6	-10.4	121
Δ-BHC	253	9.0	307	6.1	-12.1	121
heptachlor	411	22.8	494	15.6	-11.1	120
aldrin	438	17.6	517	9.2	-16.2	118
heptachlor epoxide	550	18.8	653	19.2	-12.2	119
δ-chlordane	242	8.1	287	7.7	-12.1	119
Endosulfan-I	668	23.8	798	23.5	-18.3	120
p,p'DDE	611	22.8	729	23.4	-14.0	119
Dieldrin	554	14.2	666	19.6	-18.4	120
Endrin	529	138.0	754	113.	-9.2	143
Endosulfan-II	614	33.2	849	39.5	-9.7	138
p,p'-DDD	319	14.1	372	13.9	-11.4	117
Endrin aldehyde	1080	147.0	1287	49.2	-4.8	119
Endosulfan sulfate	2472	141.0	3197	71.4	-11.3	129
p,p'-DDT	1899	66.5	2425	70.8	-19.7	128
Methoxychlor	1403	105.0	1934	51.5	-12.9	138
Mirex	h	h	h	h	h	h
					Bias Ave[g]	123%

a Average area of standard
b Standard deviation of 6 standards
c Average area of sample
d Standard deviation of 6 samples
e students "t" value where a "t" value larger than 2.6 at the 95% confidence level is statistically significant
f Bias = ave. $\frac{\text{area sample}}{\text{area standard}}$ X 100
g Average bias not including data for endrin and endrin aldehyde
h No data for mirex because of solvent impurity interferance

laboratory recoveries for Method 608 is approximately 92% or a -8% bias. This includes both extraction and packed column analytical errors. It is obvious that a bias of 23% for just the analytical step is totally unsuitable. The only conclusion is that narrow boiling range (1°C) reagents must be selected as extracting solvents if external calibrations and automated, splitless injection capillary column injection techniques are to be employed for quantitative analyses.

Retention Data Studies

The primary reason for applying capillary column systems to the analysis of environmental extracts is to utilize their unique ability to rapidly resolve complex, wide-boiling range mixtures of organic compounds. For many capillary operations, it is not unusual to observe well resolved peaks eluting from the column within seconds of one another. In reviewing data system programs routinely used to reduce packed column chromatograms, retention time windows between ±1 and 5% of the average retention time of the analyte, are normally recommended by data system manufacturers to make qualitative identifications. A retention time window here is defined as that interval of time through which the analyst is 95% sure the peak maximum for the target compound will occur. The application of packed column criteria is not suitable for capillary column work, since the ±5% retention window for a compound eluting after 20 minutes is a window 60 seconds wide. To determine reasonable retention windows for capillary column work, the retention data for the chromatograms used to generate Tables 3, 5, and other related studies were examined. Tables 12 and 13 tabulate the average retention time, RSD, and the upper and lower limits (window) at the 95% confidence level for 37 automatic and 15 manual splitless

TABLE 12

Automatic Injection Retention Data[a]

	Average Retention Time (Minutes)	Standard Deviation	Relative Standard Deviation	Min	Max	N	T
α-BHC	10.049	0.00346	0.034	10.042	10.056	37	0.8
β-BHC	10.620	0.00434	0.041	10.610	10.629	37	1.1
γ-BHC	10.791	0.00459	0.042	10.782	10.800	37	1.1
Δ-BHC	11.336	0.00502	0.044	11.326	11.346	37	1.2
Heptachlor	12.674	0.00588	0.046	12.662	12.685	37	1.4
Aldrin	13.795	0.00559	0.041	13.784	13.806	37	1.3
Heptachlor Epoxide	15.267	0.00580	0.038	15.255	15.278	37	1.4
γ-Chlordane	16.299	0.00535	0.033	16.288	16.309	37	1.3
Endosulfan I	16.913	0.00608	0.036	16.901	16.925	37	1.4
P,P'-DDE	18.061	0.00665	0.037	18.047	18.074	37	1.6
Dieldrin	18.278	0.00673	0.037	18.264	18.291	37	1.6
Endrin	19.610	0.01136	0.058	19.587	19.632	32	2.7
Endosulfan II	20.214	0.00758	0.038	20.199	20.229	37	1.8
P,P'-DDD	20.769	0.00775	0.037	20.753	20.784	37	1.9
Endrin aldehyde	21.568	0.00787	0.036	21.552	21.584	37	1.9
Endosulfan sulfate	23.484	0.00832	0.035	23.467	23.501	37	2.0
P,P'-DDT	23.802	0.00723	0.030	23.788	23.817	37	1.7
Endrin ketone	27.598	0.00762	0.028	27.583	27.614	37	1.9
Methoxy-chlor	29.087	0.00693	0.024	29.073	29.101	37	1.7
Mirex	31.568	0.00751	0.024	31.553	31.583	37	1.8

a = Retention data from 37 chromatograms collected over a 4-day period of time.
Min = Minimum Retention Time at 95 Percent Confidence Limit (minutes)
Max = Maximum Retention Time at 95 Percent Confidence Limit (minutes)
N = Number of Analyses
T = Variability of Retention Time (seconds) at 95% confidence limit

TABLE 13

Manual Injection Retention Data[a]

	Average Retention Time (Minutes)	Standard Deviation	Relative Standard Deviation	Min	Max	N	T
α-BHC	10.048	0.0071	0.070	10.034	10.062	18	1.7
β-BHC	10.622	0.0062	0.058	10.609	10.634	18	1.5
γ-BHC	10.794	0.0061	0.056	10.782	10.806	18	1.4
Δ-BHC	11.336	0.0061	0.054	11.324	11.348	18	1.4
Heptachlor	12.677	0.0075	0.059	12.662	12.692	18	1.8
Aldrin	13.798	0.0079	0.057	13.783	13.814	18	1.9
Heptachlor epoxide	15.270	0.0084	0.055	15.253	15.287	18	2.0
γ-Chlordane	16.303	0.0059	0.036	16.291	16.315	17	1.4
Endosulfan I	16.918	0.0083	0.049	16.901	16.934	17	2.0
P,P'-DDE	18.066	0.0092	0.051	18.047	18.084	18	2.2
Dieldrin	18.284	0.0078	0.043	18.269	18.300	18	1.9
Endrin	19.615	0.0099	0.050	19.595	19.635	18	2.4
Endosulfan II	20.220	0.0109	0.054	20.198	20.242	18	2.6
P,P'-DDD	20.774	0.0104	0.050	20.753	20.795	18	2.5
Endrin aldehyde	21.573	0.0113	0.052	21.550	21.595	18	2.7
Endosulfan sulfate	22.493	0.0113	0.048	23.470	23.515	18	2.7
P,P'-DDT	23.809	0.0123	0.052	23.784	23.834	18	3.0
Endrin ketone	27.605	0.0101	0.037	27.585	27.625	17	2.4

TABLE 13 (Cont'd.)

	Average Retention Time (Minutes)	Standard Deviation	Relative Standard Deviation	Min	Max	N	T
Methoxychlor	29.090	0.0108	0.037	29.068	29.112	18	2.6
Mirex	31.571	0.0102	0.032	31.551	31.592	18	2.5

a = Retention data from 18 chromatograms collected over a 6 day period of time.
min = minimum retention time at 95% confidence limit (minutes)
max = maximum retention time at 95% confidence limit (minutes)
n = number of analyses
t = variability of retention times (seconds) at 95% confidence limit

capillary injections. It is evident from these data that properly designed capillary column gas chromatographic systems can generate precise retention data from both automatic and manual splitless injections. Secondly, if the column is capable of resolving adjacent peaks by three seconds, a totally automated data system can be used to reproducibly identify complex mixtures of compounds with a high degree of reliability.

On-Column Injections

On-column injection techniques have two important advantages over splitless injections. The first, and perhaps the most significant advantage to environmental extracts, is the injection of the sample directly into the column while the latter is operated near the boiling point of the sample solvent. Under these conditions, the likelihood of analyte decomposition due to reactive surfaces and elevated temperatures normally experienced in heated splitless and splitting injectors, is minimized. The second advantage is the elimination of the quantitation problem associated with needle discrimination[8]. Needle discrimination occurs when high boiling analytes (boiling point (BP) > 300°C) are injected into a heated injector. Depending upon the injection technique used, a significant portion of the high boilers can remain in or on the exterior surfaces of the needle as it is removed from the injector, resulting in only a fraction of the high boiling analytes entering the column.) General acceptance of on-column injections by routine analytical laboratories has not progressed as rapidly as splitless injections because such operations are largely experimental in nature, require extremely delicate injection techniques, and require specialized equipment not currently automated.

On-column injections were accomplished using solvent flush techniques where the fused silica needle was flushed and filled with clean solvent. One microliter of air was drawn into the syringe, followed by 1 to 1.5 µL of sample. The sample was drawn back into the syringe so that the sample column tail was at the 1.0 µL mark on the syringe and the sample volume could thus be accurately determined. Prior to injection, the column and the injector was cooled to within 5°C of the boiling point of the solvent. The needle was wiped clean and inserted, using a clean nylon glove to guide the needle approximately 6 cm into the capillary column. The sample was rapidly injected, followed by an immediate withdrawal of the needle. Even though the injector and column were adjusted to 35°C, pentane standard injections produced chromatograms with severely tailing peaks. Table 14 lists the results of several on-column injections performed using the hexane and isooctane standards. It is evident from these data that there are no analyte area problems associated with varying the boiling point of the sample solvent as experienced with the splitless injector. Secondly, there is no evidence of endrin breakdown as indicated by excellent precision for replicate endrin injections and the absence of endrin ketone. Contrary to the manual splitless injections, these data generally exhibit excellent agreement between replicate analyses comparable to packed column performance.

During the course of the various evaluations of injection techniques, all other experimental parameters remained constant. Therefore, it is possible to examine the splitless injector data for evidence of analyte decomposition or needle discrimination by comparing the response of each pesticide in the splitless mode to that collected in the on-column mode. After discarding one set of questionable measurements, the average response (area per µL injected)

TABLE 14

Area Measurement
On-Column Injection[a]

	Area Hexane[b]	Area Isooctane[c]
α-BHC	385 (6.8)	430 (4.3)
β-BHC	280 (2.3)	297 (22.4)
γ-BHC	251 (2.9)	384 (21.3)
Δ-BHC	255 (2.2)	258 (11.1)
Heptachlor	419 (2.7)	411 (3.1)
Aldrin	414 (10.1)	392 (3.1)
Heptachlor epoxide	498 (6.9)	470 (2.9)
γ-Chlordane	208 (3.1)	199 (2.1)
Endosulfan I	580 (7.3)	564 (2.6)
P,P'-DDE	517 (2.7)	523 (2.0)
Dieldrin	493 (0.8)	487 (4.8)
Endrin	956 (1.9)	970 (3.6)
Endosulfan II	563 (2.0)	597 (10.3)
P,P'-DDD	286 (3.0)	305 (19.4)
Endrin aldehyde	914 (3.9)	999 (4.3)

was calculated for each pesticide from the data shown in Table 14. These average response data were then divided into the corresponding pesticide areas collected, using splitless injections shown in Table 3 and 5. The resulting splitless injection/on-column area ratios appear in Table 15. If no needle discrimination or analyte decomposition/formation is occurring during splitless injection, the peak area ratio should remain constant for a given set of injection parameters. If the same amount of material enters the column during splitless injections, the area ratio for splitless and on-column injections should remain constant as uncritical parameters are changed. These data show that for the unpacked injector sleeve, the area ratio for the majority of the analytes is constant for a given solvent, while for the packed sleeve the ratios are fairly constant for all the solvents. Applying an outlier test[10] to these data show that at the 95% confidence level, endrin, endrin aldehyde, and methoxychlor are outliers indicating serious quantitative problems associated with splitless injections. The RSD and means appearing at the bottom of each column in Table 15 are calculated excluding these compounds. Table 16 lists the average percent recovery for each pesticide, assuming the means determined in Table 15 to be the true value. Reviewing these data indicate that heptachlor exhibits a consistent negative bias as does DDT and mirex, indicating minor quantitative problems associated with splitless injections of these compounds.

Analysis of Product Mixtures

Included in the list of priority pollutants are several commercial products containing mixtures of closely related chlorinated compounds. These include: Toxaphene, an extremely complex mixture of polychlorinated camphene

TABLE 15

Splitless Injection/On-Column Injection Area Ratios[a]

	Empty Liner			Packed Liner		
	Pentane	Hexane	Isooctane	Pentane	Hexane	Isooctane
α-BHC	0.476	1.11	1.78	3.71	3.22	3.15
β-BHC	0.478	1.18	1.83	3.69	3.24	3.17
γ-BHC	--	--	--	--	--	--
Δ-BHC	0.486	1.19	1.91	3.87	3.32	2.90
Heptachlor	0.467	1.18	1.77	2.98	2.67	2.67
Aldrin	0.555	1.23	1.79	3.89	3.58	3.53
Heptachlor epoxide	0.543	1.27	1.83	3.56	3.22	3.35
γ-Chlordane	0.586	1.29	1.86	b	3.07	b
Endosulfan I	0.537	1.28	1.85	3.61	3.42	3.46
P,P'-DDE	0.538	1.26	1.82	3.86	3.77	3.91
Dieldrin	0.504	1.18	1.69	3.41	3.31	3.36
Endrin[c]	0.071	0.225	0.509	1.02	1.16	1.08
Endosulfan II	0.481	1.16	1.71	3.23	3.23	3.35
P,P'-DDD	0.527	1.21	1.73	3.86	3.74	3.85
Endrin aldehyde	0.654	1.53	2.06	3.71	3.42	3.34
Endosulfan sulfate	0.448	1.12	1.71	3.00	2.98	3.09
P,P'-DDT	0.370	1.03	1.57	2.94	2.98	3.09
Endrin ketone	--	--	--	--	--	--
Methoxychlor	0.252	0.811	1.32	2.58	2.72	2.97
Mirex	0.510	1.08	1.48	2.26	2.30	2.40

TABLE 15 (Cont'd.)

	Empty Liner			Packed Liner		
	Pentane	Hexane	Isooctane	Pentane	Hexane	Isooctane
Average	0.501	1.19	1.76	3.36	3.17	3.22
Relative Standard Deviation (%)	10.4	6.4	6.5	15.2	12.2	12.5

a = $\frac{\text{area of splitless injection}}{\text{area/}\mu\text{L of on column injection}}$

b = no on-column data for pentane, see text

TABLE 16

Splitless Injection Recovery for Test Compounds

	Empty Liner			Packed Liner			Ave. %	
	Pentane	Hexane	Isooctane	Pentane	Hexane	Isooctane	Recovery	RSD
α-BHC	95	93	101	110	102	98	99.8	6.1
β-BHC	95	99	104	110	102	98	101	5.2
γ-BHC	—	—	—	—	—	—	—	—
Δ-BHC	97	100	109	115	105	90	103	8.7
Heptachlor	93	99	101	89	84	83	92	8.2
Aldrin	111	103	102	116	113	110	109	5.1
Heptachlor epoxide	108	107	106	106	102	104	106	2.1
γ-Chlordane	117	108	106	—	108	—	—	—
Endosulfan I	107	108	105	107	108	107	107	1.0
P,P'-DDE	107	106	103	115	119	121	112	6.7
Dieldrin	101	99	96	101	104	104	101	3.0
Endrin	14	19	29	30	37	34	27	33
Endosulfan II	96	98	97	96	102	104	99	3.4
P,P'-DDD	105	102	98	115	118	120	110	8.4
Endrin aldehyde	131	129	117	110	108	104	117	9.7
Endosulfan sulfate	89	94	97	89	94	96	93	3.7
P,P'-DDT	74	87	89	88	94	96	88	8.8
Endrin ketone	—	—	—	—	—	—	—	—
Methoxychlor	50	68	75	77	86	92	75	20
Mirex	102	91	84	67	73	75	83	16

derivatives; Aroclors 1016, 1221, 1232, 1242, 1248, 1254, and 1260, complex mixtures of polychlorinated biphenyls; and chlordane, a mixture primarily of the isomers of chlordene and chlordane, heptachlor, and nonachlor. Capillary column separations of these mixtures provided a significant improvement in the ability to resolve the individual components over packed column gas chromatography. Close examination of the data, however, shows that for routine analyses, the capillary column provided too much information as lengthy complex chromatograms were obtained. Further complicating the capillary column analyses, significant differences were noted between various production batches of each product resulting in a moderate degree of uncertainty in qualitative identifications and potentially serious quantitative errors if calculations are based upon single peaks. For such mixtures, assuming no interferences, packed column gas chromatography affords an advantage over capillary columns, since sufficient resolution is obtained to identify the parent product through the recognition of a series of peaks with correct relative intensities at known retention times. For packed column analysis, the multitude of coeluting compounds tend to "average out" small variations in product batches resulting in accurate qualitative and quantitative analyses.

SECTION 3

CONCLUSIONS AND RECOMMENDATIONS

The results of this evaluation show that the application of capillary column techniques to the analysis of priority pollutants can afford a distinct advantage over most conventional packed column technology. Dilute complex mixtures of coextractables can be simultaneously analyzed using temperature programmed capillary columns and most popular ionization detectors. To obtain a similar degree of separation and conformation the packed column analytical protocol currently described in the 600 series methods requires sample clean-up by column chromatography followed by one or more injections into packed columns operated under isothermal or limited temperature programmed conditions especially when the analytes are near the instrument limit of detection. For the analysis of individual analytes, the capillary systems clearly provide these advantages; however, for complex formulations or technical mixtures such as toxaphene and Aroclors packed columns greatly simplify the analysis.

Even though the glass and fused silica surfaces were shown to be inert through the use of polarity test mixes, the splitless injector caused endrin to degrade unless a continuous extraordinary deactivation program was undertaken. For the NIPDWR (2) methods and methods 608 and 625 the quality control, calibration and interference sections should be revised so that the analyst will be alerted to the possibility of false negative results for endrin and false positive data for endrin aldehyde.

The priority pollutants include compounds with neutral, acetic and basic chemical properties. Of all the columns tested, a 30m, 0.32 mm ID, 0.25 μm df fused silica column with a polarity similar to polymethyl (5% phenyl) siloxane afforded the best separations of such mixtures within a reasonable period of time. The life expectancy of bonded phase columns significantly outperformed non-bonded phase columns. The substrate bleed signal for bonded phase columns generated during temperature programs was lower than that of non-bonded phase columns with similar polarities providing the advantage of lower instrument sensitivity limits when bonded phase columns are used.

The inclusion of 4,4'-dibromobiphenyl in each sample and column polarity test mixtures appears to be convenient means of predicting early column failure, especially for electron capture systems.

If splitless injections are to be used for quantitative analyses, then injections must be made using autosamplers in order to generate precision data comparable to that of manual packed column techniques. The precision of autosampler data appears to be adversely affected as the boiling point of the sample solvent decreases. From the limited studies performed herein, the best performance is obtained using solvents that exceed the boiling points of pentane and methylene chloride. For accurate analyses the sample and the standard must be contained in exactly the same solvent if external calibration techniques are to be used. When samples and standards are contained in dissimilar solvents then internal standard calibrations must be used to compensate for variable amounts of analytes entering the column during the splitless injection routine. If splitless capillary systems are to be used in place of normal packed column operations, then it is imperative that the

calibration and procedure sections of the methods be updated to reflect these observations.

On-column injection techniques appear to resolve all the problems identified in this study associated with splitless injections. On-column injections consistently provided precise qualitative and quantitative data for organohalide pesticides and demonstrated no signs of analyte decomposition or variable amounts analyte entering the column as solvents are varied.

This is a continuing study and an effort will be made to determine if the problems encountered with splitless injections are inherent to the injector design chosen for this study or if they are associated with the mechanism involved in sample delivery.

As the opportunity arises, other automated splitless injectors and on-column injectors will be evaluated.

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